

reaction was performed at room temperature for 2 h. After the reaction was completed, the reaction solution was filtered, and then a small amount of sodium carbonate aqueous solution (50 mg Na<sub>2</sub>CO<sub>3</sub>, 4 mL) was added to adjust the pH to about 8-9. A small amount of water and methanol were added and the mixture was lyophilized and purified by the preparative liquid chromatography to obtain the target product (white solid, 80 mg, purity >99%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 8.20 (d, J=7.6 Hz, 2H), 7.76~7.79 (m, 1H), 7.65~7.71 (m, 4H), 7.42~7.51 (m, 3H), 7.27 (s, 2H), 4.89~4.97 (m, 2H), 3.75 (d, J=2.8 Hz, 1H), 3.38 (d, J=2.8 Hz, 1H), 3.32 (s, 1H), 2.73~2.82 (m, 2H), 2.31~2.40 (m, 2H), 2.22~2.26 (m, 2H), 1.81 (t, J=14 Hz, 1H), 1.23~1.30 (m, 1H), 1.10~1.11 (m, 1H), 0.97 (s, 3H), 0.82 (d, J=6.4 Hz, 3H), 0.65 (d, J=6.8 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 167.2, 164.3, 150.3, 141.9, 135.0, 131.9, 131.7, 130.0, 129.8, 129.4, 128.9, 128.7, 127.6, 126.9, 90.7, 75.7, 63.7, 62.9, 60.5, 59.8, 54.7, 53.3, 36.1, 28.6, 25.3, 23.8, 17.3, 17.2, 16.8, 15.0. <sup>31</sup>P-NMR (400 MHz, CDCl<sub>3</sub>): δ -2.1. MS calcd for C<sub>35</sub>H<sub>35</sub>O<sub>2</sub> P(M<sup>-</sup>): 677.2, found 627.2.

Example 11: Detection of In Vitro Antitumor Activity of Small Molecule Compounds CK21S-001, CK21S-001-b, CK21S-002, CK21S-002-b, CK21S-003, CK21S-003-b, CK21S-004, CK21S-004-b, CK21S-005, CK21S-005-b, CK21S-006, CK21S-006-b, CK21S-007, CK21S-008, CK21S-009 and Triptolide

[0151] Tumor cells included AsPC-1 (human pancreatic cancer cell), PC-3 (human prostate cancer cell), and SK-OV-3 (human ovarian cancer cell). The source and culture medium of the cells were shown in Table 1 below.

TABLE 1

Tumor cell information				
Cell line	Supplier	Cat#	Tumor cell type	Medium
AsPC-1	ATCC	CRT-1682	human pancreatic cancer cell	RPMI 1640 + 10% FBS + 1X PS
PC-3	ATCC	CRL-1435	human prostate cancer cell	F-12K + 10% FBS + 1X PS
SK-OV-3	ECACC	91091004	human ovarian cancer cell	McCoy's 5a + 10% FBS + 1X PS

[0152] Triptolide was used as a positive control; the working concentrations of the test drug were 1 μM, 0.33 μM, 0.11 μM, 0.037 μM, 0.012 μM, 0.004 μM, 1.4 nM, and 0.46 nM. After the tumor cells were revived, they were resuspended in the corresponding complete medium, placed in 5% CO<sub>2</sub>, and cultured at 37° C. The tumor cells at the logarithmic growth stage were suspended in the corresponding complete medium to adjust the cell concentration. 90 μL of cell suspension was added into each well. The number of each type of cells was shown in Table 2 below. The cells were incubated at 37° C. in 5% CO<sub>2</sub> overnight. Each concentration of the test drug was added and the cells were further cultured for 48 hours. Finally, Promega CellTiter-Glo Luminescent Cell Viability Assay Kit was used for detection.

TABLE 2

Cell plating density	
Cell line	Cell number/well (96 well plate)
AsPC-1	5000
PC-3	5000
SK-OV-3	3000

[0153] The IC<sub>50</sub> values for in vitro antitumor activity of Compounds CK21S-000, CK21S-001-b, CK21S-002, CK21S-002-b, CK21S-003, CK21S-003-b, CK21S-004, CK21S-004-b, CK21S-005, CK21S-005-b, CK21S-006, CK21S-006-b, CK21S-007, CK21S-008, CK21S-009 and triptolide were shown in Table 3. All compounds had in vitro antitumor activity, which was not much different from that of the positive control triptolide.

TABLE 3

In vitro antitumor activity as IC <sub>50</sub> (μM)			
Relative IC <sub>50</sub> (μM)	AsPC-1	PC-3	SK-OV-3
CK21S-001	0.028	0.022	0.042
CK21S-001-b	0.030	0.029	0.048
CK21S-002	0.071	0.056	0.070
CK21S-002-b	0.042	0.042	0.057
CK21S-003	0.014	0.018	0.028
CK21S-003-b	0.020	0.021	0.029
CK21S-004	0.038	0.023	0.118
CK21S-004-b	0.096	0.045	0.129
CK21S-005	0.040	0.030	0.041
CK21S-005-b	0.042	0.035	0.040
CK21S-006	0.035	0.031	0.041
CK21S-006-b	0.036	0.034	0.045
CK21S-007	0.049	0.043	0.050
CK21S-008	0.035	0.030	0.038
CK21S-009	0.030	0.027	0.032
Triptolide	0.014	0.014	0.016

### Example 12

Detection of In Vitro Immunosuppressive Activity of Small Molecule Compounds CK21S-001, CK21S-001-b, CK21S-002, CK21S-002-b, CK21S-003, CK21S-003-b, CK21S-004, CK21S-004-b, CK21S-005, CK21S-005-b, CK21S-006, CK21S-006-b, CK21S-007, CK21S-008, CK21S-009 and Triptolide

[0154] Preparation of Mouse Spleen Lymphocyte

[0155] BALB/c mice were sacrificed by spinal dislocation, and their spleen was aseptically taken out. A single cell suspension was prepared and adjusted to the required concentration.

[0156] Cell Proliferation Test

[0157] A suspension of 4×10<sup>6</sup> cells/ml spleen lymphocytes was routinely prepared and 100 μL of cells was added into each well of a 96-well plate. 50 μL test sample in different concentrations was added and ConA (final concentration 5 μg/mL) was added to induce T lymphocyte activation and proliferation, or LPS (final concentration 10 μg/mL) (50 μL) was added to induce B lymphocyte activation and proliferation. The corresponding positive control and non-stimulating background control were set. The cells were cultured in 37° C., 5% CO<sub>2</sub> incubator for 48 h. 0.25 μCi <sup>3</sup>H-thymidine was introduced and the cultivation was completed after 8 h.